The Journal of HEREDITY

THREE GENERATIONS OF POLYPLOID SALAMANDERS
(SEE PAGE 7)

Somatic Reduction in Trillium

Two Genes for Tongue-Twisting

Mustard Gas and Meiosis

A New Gene for "Shrunken Endosperm"

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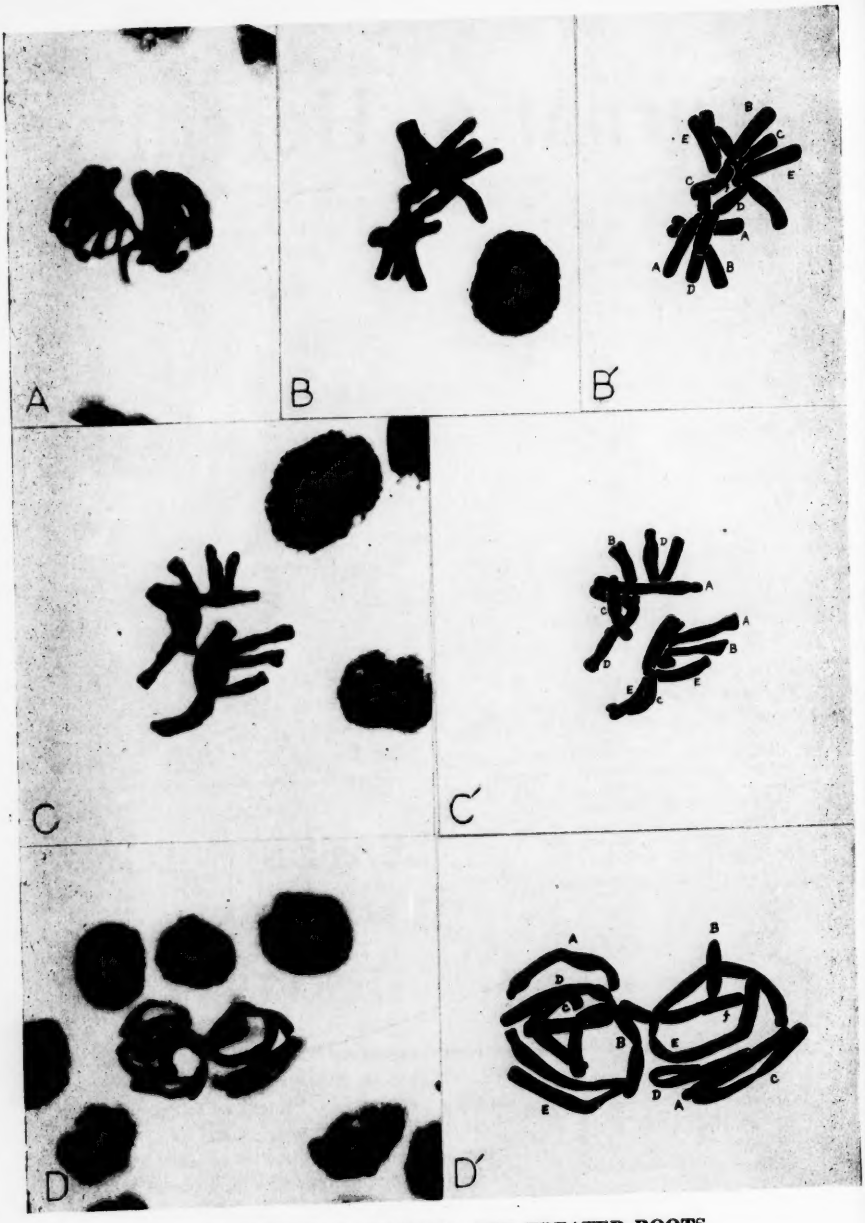


REDUCTIONS IN CONTROL AND TREATED ROOTS

Figure 1

Reduction-divisions in *Trillium ovatum* roots, both in controls and in roots treated with sodium nucleate. *A*—segregating prophase. *B* and *B′*—(5:5) non-homologous reduction in untreated root. *C* and *C′*—(5:5) non-homologous reduction in treated root. *D* and *D′*—(5:5) homologous reduction in treated root. This plant has an extra fragment chromosome, *f*. Magnification *ca* 800×.

SEGREGATION AND REDUCTION IN SOMATIC TISSUES

II. The Separation of Homologous Chromosomes in Trillium Species

G. B. WILSON AND K. C. CHENG*
Department of Botany, University of Wisconsin

TREATMENT with solutions of sodium nucleate of tissues undergoing mitosis induces, amongst other departures from normal, a certain number of reduction type divisions as reported by Huskins.[2,3] Reduction-divisions have also been found in *Allium* root tips after treatment with phosphates (Galinsky, unpub.) as well as in untreated roots from onions which have been stored for several months and in untreated Trillium root tips near the end of their root formation period.

The theoretical and practical implications of these reduction-divisions, both natural and induced, have been discussed in detail by Huskins[4] in the first paper of this series. The initial work on *Allium cepa* left unanswered the question of whether or not homologous chromosomes are preferentially separated in these segregations, owing to the fact that it is difficult to distinguish homologues with any degree of certainty in that species. This is the primary question to be considered in the present paper.

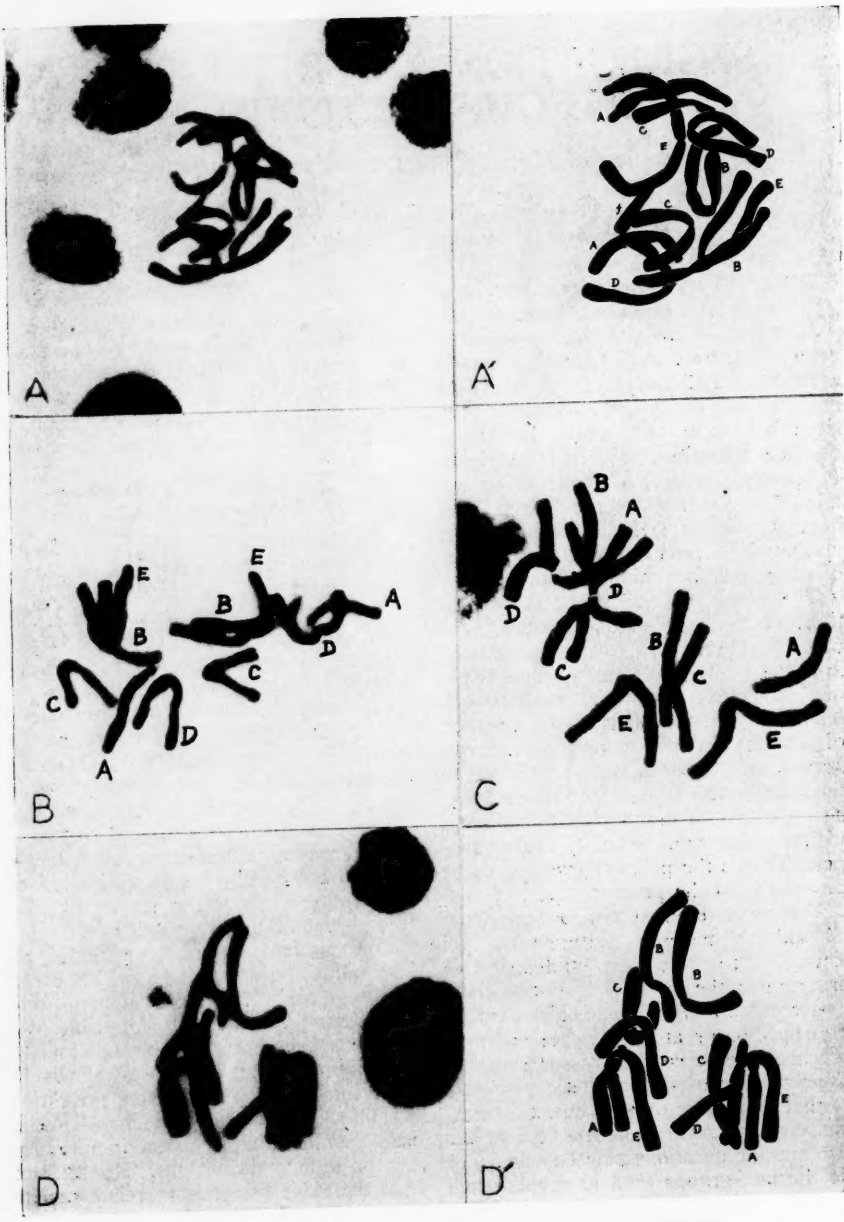
The most common observation, from all sodium nucleate treatments in all eleven plant genera so far studied in this program is the separation of the chromosomes into two groups. This separation may occur at any degree or stage of chromosome condensation from that characteristic of early mitotic prophase to that of mitotic or even meiotic metaphase. With regard to these separations two specific questions may be asked: 1) do the two groups tend to equality and 2) are homologues separated with greater than random frequency? The *Allium* data presented by Huskins[4] gave a positive answer to the first question and it is confirmed by the Trillium data presented below. They give, in addition, a positive answer to the second question.

Materials, Methods and Observations

The fact that each of the five chromosomes of Trillium species can be recognized with considerable certainty makes this genus, at first sight, most suitable for the study of the behavior of homologues. In practice, however, we have been hindered by the fact that its response to treatment with sodium nucleate is usually very slight and statistically significant numbers are therefore difficult to obtain. In addition there is the difficulty that the chromosomes are so large in relation to the cell that anaphase separations are often insufficiently wide to allow accurate determination of the number of chromosomes segregating to each pole. All cases considered doubtful on this basis have been excluded, as have all those separations which could plausibly be interpreted as due to pressure during preparation of the slides.

It has been necessary to accumulate the data presented from a relatively large number of experiments, not all of which were primarily designed for the purpose of obtaining the maximum number of reduction-divisions. No attempt will therefore be made to relate results

*Project Associate (now Professor of Botany, Michigan State College, East Lansing, Michigan) and Research Assistant respectively in the Program in Cytology supported in part by the Research Committee of the Graduate School from funds supplied by the Wisconsin Alumni Research Foundation. The authors are indebted to Professor C. Leonard Huskins for help throughout the study, to Dr. K. Patau for checking and extending the statistical analysis, and to Mr. Alfred Owczarzak for the photomicrography.



HOMOLOGOUS AND NON-HOMOLOGOUS REDUCTIONS

Figure 2

Reduction-divisions in *Trillium ovatum* roots treated with sodium nucleate. *A* and *A'*—(5:5) homologous reduction; extra fragment present. *B*—(5:5) homologous reduction. *C*—(5:5) non-homologous reduction. *D* and *D'*—(6:4) reduction with all homologues except the *B* chromosomes in separate groups. It is possible that the two *B* chromosomes may still be in process of separating. Magnification *ca* 800×.

to either time or dosage. However, most of the data on *Trillium sessile* came from treatment of excised roots with 4% sodium nucleate for 3-6 days at 2°C and those of *T. ovatum* and *T. erectum* from treatments of excised roots with 2% and 4% for 24 and 42 hours at 4-6°C.

Illustrations of typical "somatic reduction" divisions from *T. ovatum* root tips are given in Figures 1 and 2. While those which are squashed out to such an extent as to seriously damage the cell may be considered to be open to other interpretations, experience leads us to believe that they are vital rather than accidental separations. Few of these have been included in the numerical data presented, however. Those illustrated are presented primarily to show clearly the morphology of the chromosomes.

In Table I, column 5, is given the number of equal separations out of the total of reductional metaphases (column 2). It is clear in all three species, despite the low number of observations in *T. erectum*, that there are over twice as many equal (5:5) separations as expected on a completely random basis. When it is also considered that most of the other separations are 6:4, we can justifiably conclude that there is a strong tendency to equal separation. Prophase separations such as that shown in Figure 1*A* are not included in the Table since in them the homologues cannot readily be identified.

Table I also gives an analysis of the 5:5 groups with reference to segregations of complete sets. With a random distribution of homologues, approximately 1 in 8 of the 5:5 separations would be expected to segregate complete sets or genomes. This expectation is greatly exceeded in *T. sessile* and *T. ovatum* but not in *T. erectum*. This, however, does not indicate differences between these species since the number of observations in *T. erectum* is too small for significance.

In view of the possibility (which was raised by observations, unpublished, on *Crocus zonatus*) that different chromosomes might have different frequencies of homologous segregation, those slides of *Trillium ovatum* and *T. erectum* which were still available were re-examined. Table II shows: 1) Of the 270 pairs of homologous chromosomes, 194 or 72% were segregated, while on a random basis only 135 were to be expected ($\chi^2_1 = 52$, $P < 10^{-10}$). 2) That proportion seems to be the same in all chromosomes. Since some of the chromosomes are easier to identify than others this homogeneity stresses the impossibility of explaining the excess of homologous segregation by faulty classification. 3) The percentage of segregating chromosome pairs is practically the same in 5:5 as in 6:4 and 7:3 separation.

TABLE I. Reductional and Non-Reductional Metaphases in treated root-tips of *Trillium* species.

Species	Total Metaphases: Red.	Total Metaphases: Non-Red. (i.e. normal mitoses)	Total Metaphases: % Red.	5:5 Distributions in reductional metaphases: total	5:5 Distributions in reductional metaphases: homologous
T. sessile					
obs.	35	879	3.9	21	14
exp.				8.6	2.7
X^2				23.7	54.3
P				10^{-6}	$< 10^{-6}$
T. ovatum					
obs.	68	1,867	3.5	37	12
exp.				16	4.6
X^2				36.6	13.6
P				$< 10^{-6}$	0.0002
T. erectum					
obs.	19	370	5.1	10	1
exp.				4.7	1.3
X^2				8.0	
P				0.0047	

P values read from X^2 table of K. PATAU, *Z.I.A.V.* 80: 558-564. 1942.

TABLE II. Segregation of individual chromosome pairs in *Trillium ovatum* and *T. erectum*.

Chromosomes	Separation type of nucleus	Homologues: To same side	Homologues: Segregated	X^2
A	5:5	12	22	0.839
	6:4 } 7:3 }	7	13	0.485
B	5:5	7	27	0.984
	6:4 } 7:3 }	4	16	0.634
C	5:5	9	25	0.052
	6:4 } 7:3 }	7	13	0.485
D	5:5	12	22	0.839
	6:4 } 7:3 }	6	14	0.040
E	5:5	7	27	0.984
	6:4 } 7:3 }	5	15	0.089
				5.431
All Chromosomes	5:5	47	123	
	6:4 7:3	29	71	0.064
Total		76 or 28.1%	194 or 71.9%	

Total heterogeneity ..... $X^2_0 = 5.431$

Heterogeneity between 5:5 and 6:4 plus 7:3 ..... $X^2_1 = 0.064$; $P = 0.8$

Heterogeneity between chromosomes .. $X^2_8 = 5.37$; $P = 0.7$

Discussion

The data given above together with those from *Allium*[4] show that in the induced "somatic meiosis" two phenomena occur with much greater than random frequency. They are: 1) separation of chromosomes into two numerically equal groups, and 2) segregation of homologues. The difficulty of making an objective analysis of the numerical separations when the groups of chromosomes are not widely separated is obvious. We feel confident, however, that our scoring has not been greatly biased. Independent evidence of this is found in the second fact that homologues are segregated with greater than random frequency. If the preponderance of 5:5 separations were accidentally determined or subjectively influenced to any extent, this would coincidentaly reduce the frequency with which complete genomes were segregated. This in itself proves that the great excess of 5:5 separations is real.

The fact that "somatic meiosis" sometimes occurs in untreated controls, at least suggests that the operating force is inherent in the chromosomes or nucleus and is either unmasked by suppression of competing factors or brought up to the threshold of expression by the treatment or, in the case of controls, it could perhaps be by unusual accumulation of substances similar to that used in the experiment. The data of Table II showing similar proportions of segregating homologues in the 6:4 and 7:3 separations as in the 5:5 are evidence that the pairs of chromosomes, not the nucleus as a whole, are the units acted upon.

In Crocus, it has been noted by L. Steinitz and R. E. Duncan that somatic segregation is associated with a type of "distance conjugation." In Trillium there is no evidence of previous pairing at any time either of an association or "distance conjugation" type. The segregation can, however, be interpreted as similar in certain respects to that of normal gonocyte meiosis in some organisms (see reference 4), but by a process that is not as well or as effectively organized, though for that matter it is no more poorly organized than meiosis in many hybrids with "genic sterility" (as distinct from chromosome sterility[1]).

Summary

Evidence is presented from experiments on three species of Trillium that reduction- divisions resulting from treatment, with sodium nucleate, or root tips undergoing mitosis tend not only to separate the chromosomes into two groups approximately equal numerically but also to separate homologues and thus bring about regular genetic segregation with much greater frequency than would be expected if the distribution of the ten chromosomes were a random process.

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1. Dobzhansky, T. *Genetics and the Origin of Species.* Columbia Univ. Press. 2nd ed. revised. 1941.
2. Huskins, C. L. *Amer. Nat.* 81:401-434. 1947.
3. ———— *Nature* 161:80-89. 1948.
4. ———— *J. Hered.* 39:310-325. 1948.

✦

Children Should Be Told the Truth!

The Rev. Anselm Leaby, in a sermon at New York's St. Patrick's Cathedral, criticized parents who hush their children or turn to the stork when asked about birth. "Tell the child: every little baby comes from God to its mother; lives with mother several months; and mother suffers much in bringing the little one into the world. Don't give him the stork story. Later he learns the truth, sometimes in the gutter, and he suspects there may be something wrong with human procreation." Wash. *News,* 6 Dec. 1948.

THREE GENERATIONS OF POLYPLOIDS IN AMBYSTOMID SALAMANDERS*

R. R. Humphrey and G. Fankhauser

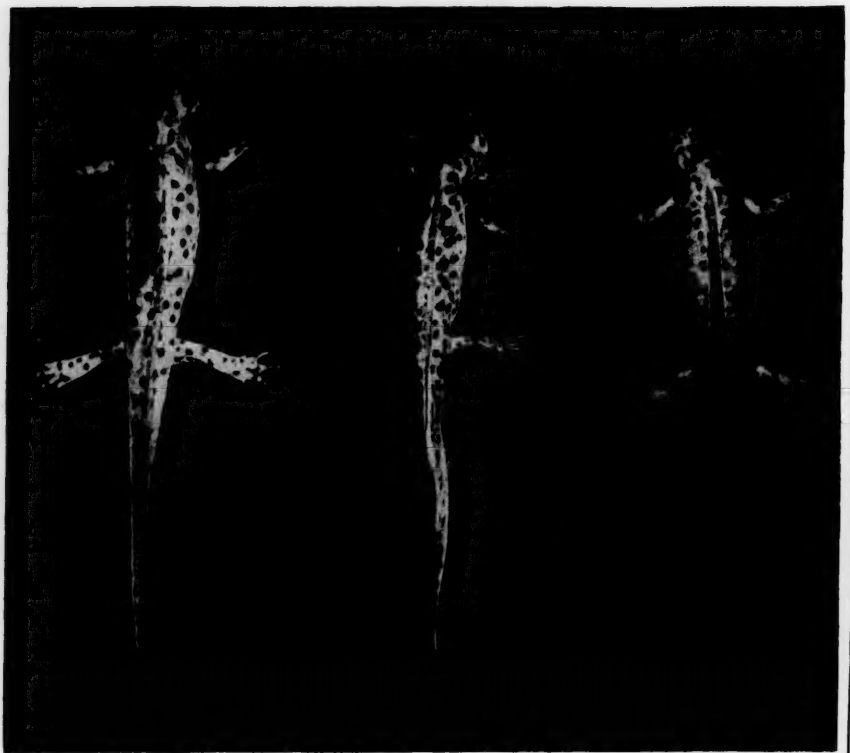
ALTHOUGH aberrations in chromosome number in salamanders, both spontaneous and induced, have received much attention during the past decade, the polyploids identified (*3n, 4n, 5n*) have in most instances either failed to attain sexual maturity, or have been found sterile or incapable of reproduction. The three generations of laboratory-reared polyploid females pictured in Figure 3 therefore constitute a notable and unique family group unlikely to be duplicated in any other vertebrate species for some time to come. The three generations, in addition to their polyploidy, are further unusual in that they alternate in chromosome number, the female of the second generation (Figure 3, *B*) being a tetraploid (*4n*) while her mother (*A*) and daughter (*C*) are triploids (*3n*). The history of this unusual family is presented briefly in the form of a simplified pedigree in Figure 4.

The female of the first polyploid generation (*A*) is one of several triploids induced by refrigeration of the freshly laid eggs of a diploid female in 1944; the procedure followed was that described by the authors in an earlier paper.[4] This diploid female, like its triploid daughter, was of dark color. It was of mixed ancestry, its mother being a Mexican axolotl (*Siredon*—or *Ambystoma—mexicanum*) of the white or partial albino strain and its father a hybrid produced by mating a male of this same strain with a similar female carrying a graft ovary from an *Ambystoma tigrinum* donor. Triploid *A* thus owes her color pattern to this donor (a "great-grandmother" who died as an embryo after furnishing the graft mentioned) but is more largely of white axolotl descent (five-sixths, theoretically, since her father was a white axolotl; see Figure 4).

In the axolotl, triploid females are not completely sterile,[7] although some may fail to mate and spawn, probably due to the very small number of eggs brought to maturity in their ovaries. Female *A* is credited with the largest known spawning of any triploid (614 eggs); she was about 25 months of age at the time, and the spawning was her first, since triploid axolotls (unlike many diploid females) do not as a rule spawn at one year.

Because of the lack of polyploid males capable of reproduction, polyploid females have always been mated with diploid males (Figure 4). With a triploid female, such a mating ($3n \times 2n$) results in offspring the great majority of which have chromosome numbers intermediate between *2n* and *3n* (unpublished data of the authors). Only very rarely are such hyper-diploid larvae capable of long survival beyond the hatching stage, and no offspring at all of this type from female *A* have been reared. In each of her two spawnings, however, a few tetraploids (*4n*) have been found, and five of these have lived for two years or more. Presumably, these tetraploids result from the occasional production by the triploid female of unreduced or triploid eggs which are fertilized by normal (haploid) spermatozoa. The meiotic irregularities leading to the formation of such triploid eggs are not known as yet. However, it is interesting to note that the tetraploid offspring of female *A* included approximately equal numbers of white and

*Department of Anatomy, University of Buffalo, Buffalo, New York; and Department of Biology, Princeton University, Princeton, New Jersey. The authors wish to express their appreciation to Mr. Melford D. Diedrick, Director of Medical Illustration at The University of Buffalo, for invaluable assistance in the preparation of the figures.



THREE TRIPLOID AND TETRAPLOID GENERATIONS

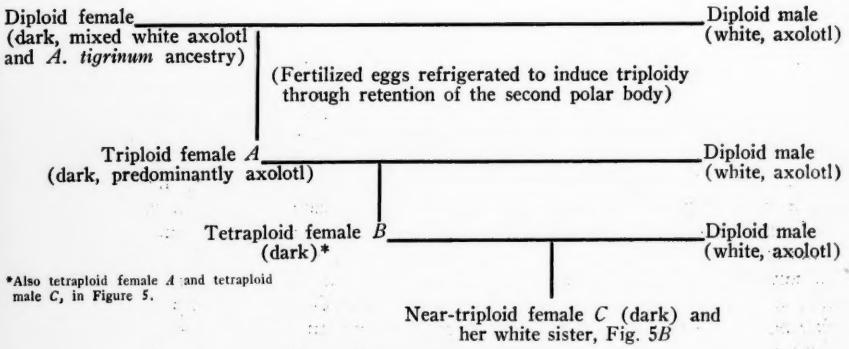
Figure 3

Three generations of polyploid female salamanders, largely white Mexican axolotl in descent but deriving their spotted color pattern from an *Ambystoma tigrinum* ancestor. *A*—Triploid (3*n*) resulting from the refrigeration of eggs of a diploid (2*n*) of mixed ancestry mated with a diploid white axolotl male; photographed at 44 months. *B*—Tetraploid (4*n*) daughter of *A* from a mating with a diploid white axolotl male; photographed at 18 months. *C*—Near-triploid daughter of *B* from a mating with a diploid white axolotl male; photographed at 17 months. All ×0.3.

dark animals of both sexes,—i.e., they offer definite evidence of segregation of the genes for color and of the differential segments of the *W* and *Z* sex chromosomes in the course of the modified meiosis.*

One of the tetraploid daughters of *A* is shown in Figure 3*B*. This animal had a dark color pattern not greatly unlike that of her mother. Unlike the latter, however, she attained sexual maturity before the end of her first year; after spawning at the age of 11½ months and again in the following spring at the age of 24 months, she died of unknown cause when 28 months old. She had

*In the ambystomid salamanders the female rather than the male is heterogametic; the sex-chromosome constitution of the female is therefore designated as *ZW*, and that of the male *ZZ*. Evidence for this mode of sex determinatiton was obtained from a study of the progeny of females experimentally reversed to males (see Humphrey, *Am. Jour. Anat.* 76. 1945).


Diploid female
(dark, mixed white axolotl and A. tigrinum ancestry)
Diploid male
(white, axolotl)
(Fertilized eggs refrigerated to induce triploidy through retention of the second polar body)
Triploid female A
(dark, predominantly axolotl)
Diploid male
(white, axolotl)
Tetraploid female B
(dark)*
Diploid male
(white, axolotl)
*Also tetraploid female A and tetraploid male C, in Figure 5.
Near-triploid female C (dark) and her white sister, Fig. 5B

ANCESTRY OF POLYPLOIDS

Figure 4

Simplified pedigree to show the relationships of the polyploids pictured in Figures 3 and 5.

grown very little in the period after the photograph (Figure 3) was taken (at 18 months) and would probably never have equalled her mother in size. Her white tetraploid sister (Figure 5*A*), on the other hand, was almost the same length at two years as her mother at four, and was also somewhat less slender of body.

These tetraploid females, like their triploid mother, could be mated only with diploid males (see Figure 4). Such matings ($4n \times 2n$) result in progeny with chromosome numbers ranging from 32 to 44, although the majority approximate the triploid number, 42.[5] A relatively high percentage of these young (as compared with the offspring of triploids) prove capable of survival, even though some larvae with exactly 42 chromosomes have been found nonviable, exhibiting the same abnormalities as are encountered in the hyperdiploid progeny of triploids (circulatory deficiencies, ascites, edema, etc.). Tetraploid *B*, mated with a white diploid male, produced both dark and white offspring, male and female, in approximately equal numbers. This suggests that she carried only one dominant gene for the dark color pattern (*Dddd*) and that her sex-chromosome constitution was *WZZZ*.

The third-generation animal shown in Figure 3*C* is one of the numerous viable offspring of tetraploid *B*. It has a chromosome number of about 41. When photographed at 17 months of age its pattern of spotting was somewhat less distinct than that in its mother or grandmother; this, however, is probably of little significance, since the coloration even of siblings usually shows considerable variation, and the contrast between spots and background color in this animal may increase with age. A white sister of this dark female, photographed at the same age, is shown in Figure 5*B*; this white (whose chromosome number, probably close to 42, has not yet been accurately determined), is unusually large for its age, and bids fair to become the largest polyploid ever reared by the authors.†

The possibility of continuing the line of polyploids here carried through three generations depends in considerable part upon the chance occurrence of viable $4n$ females among the offspring which may later be obtained from the triploid or near-triploid females of the third generation (animals such as 3*C* and 5*B*). These latter animals have not yet attained maturity. Most of their progeny, as with offspring of any triploid female, may be expected to be hyper-diploids

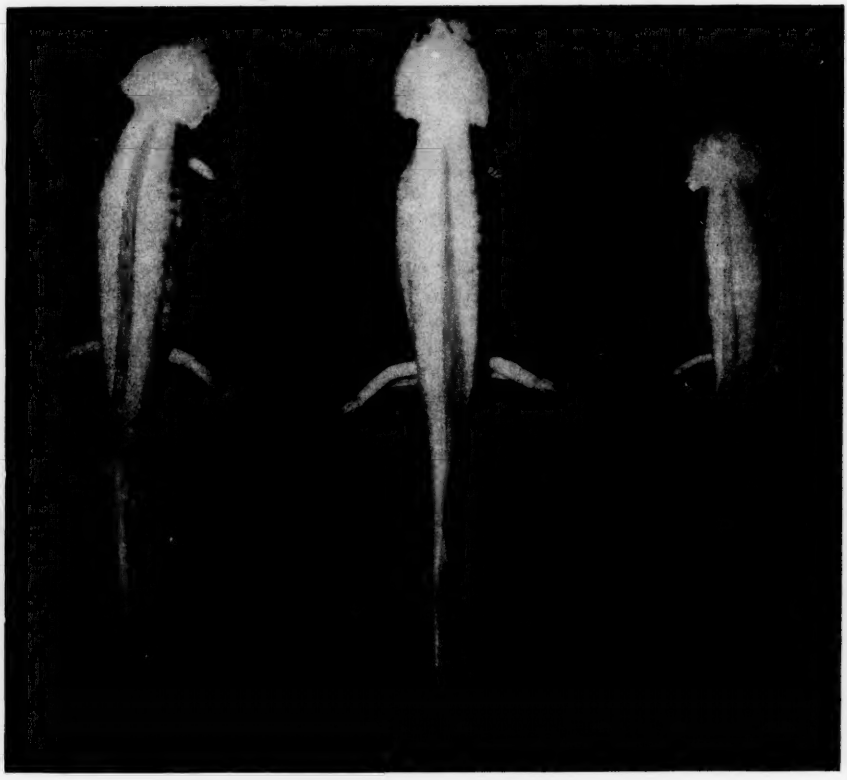
†Another three-generation series of polyploid females comparable to that described has been obtained with animals exclusively of white axolotl ancestry.

incapable of survival, or, if surviving, unlikely to reproduce. The chance failure of an egg of one of these triploids to reduce its chromosome number (thus producing a viable tetraploid female) will therefore offer the best possibility of continuing a line of descent which would otherwise end with the usual nonviable offspring of triploid females.

Thus far no polyploid male has reproduced, but it is hoped that among the several triploid or near-triploid animals of this sex now being reared some may be found capable of doing so. Other triploid males previously reared have emitted spermatophores which were either sterile or carried only relatively small numbers of spermatozoa, and the females mated with such males did not spawn. The two tetraploid males thus far reared to ages of over two years, unlike their tetraploid sisters, are quite subnormal in size and vigor, and still have cloacae of juvenile type. The larger, a white animal, is shown in Figure 5*C*. Certain tetraploid females have similarly proven incapable of survival to sexual maturity or have died at one to three years of age without reproducing. It is possible that such females, as well as the two 4*n* males reared, may have a chromosomal imbalance underlying their failure to attain the maximum size and vigor exhibited by certain tetraploid females. The small number of males thus far dealt with makes it impossible to state that tetraploids of this sex will *always* be subnormal and sterile merely as the result of their male constitution or genotype. Triploid males, or near-triploids, are of course often of more or less normal size even though as yet they, too, have failed to reproduce. In general, however, the *average* polyploid, male or female, would appear to be inferior to the average diploid of the same ancestry, even though occasional polyploid females (such as *A* and *B* in Figure 5) have proven exceptional as to size and vigor.

The relatively high reproductive capacity of polyploid females in ambystomid salamanders is in contrast with the sterility or near-sterility of such females in other species. Fischberg[6] who has made an extensive study of triploidy in *Triton alpestris,* states that the ovaries in triploid females of that species usually contain only an occasional growing oocyte (auxocyte) and that these cells later degenerate instead of developing to maturity. He found the ovaries of the three-year triploid no better developed than those of younger ones, and concluded that these animals would remain permanently sterile. The ovaries of triploids of the American newt, *Triturus viridescens,*[3] would appear to be very similar to those of *Triton alpestris,* although the difficulty of maintaining these newts in the laboratory after metamorphosis has thus far prevented a fully satisfactory study of older polyploids. Spontaneous triploidy is of relatively frequent occurrence in another American salamander, *Eurycea bislineata,* according to Fankhauser[2] and Michalski,[8] but to the writers' knowledge, fully adult triploid females of this species have never been identified and the character of their ovaries ascertained.

The triploid males of other species of salamanders may possibly be superior in reproductive capacity to those of the axolotl. Fischberg[6] states that the testes of *Triton alpestris* triploids contain numerous mature spermatozoa, and figures a testis of apparently normal structure from a two-year animal. He states further that the triploid males engage in the usual courtship when placed with diploid females, but that they are sometimes treated as females by both diploid and other triploid males. He does not record the emission of normal spermatophores by triploid males, however, and apparently obtained no offspring from them. The reproductive capacity of the adult *Triton taeniatus* male triploid described by Bőők[1] is likewise uncertain, although in this animal, too, mature spermatozoa were found in the testes. No adult triploid males of *Triturus viridescens* or *Eurycea bislineata* have been reported, despite the frequency of spontaneous polyploidy in these species. All in all, there would be little prospect for a three-generation series of male polyploids comparable to that here



WHITE POLYPLOIDS

Figure 5

White or partial albino polyploids descended from triploid female *A* of Figure 3. *A*—Tetraploid daughter of *A* and sister of *B* in Figure 3; photographed at 29 months. *B*—Triploid (or near-triploid) daughter of *B* and sister of *C* in Figure 3; photographed at 17 months. *C*—Male tetraploid, son of triploid *A* and brother of tetraploid females *B* Figure 3 and *A* Figure 5; photographed at 29 months. All ×0.3.

described for females.

It is obvious that if polyploidy results in sterility or near-sterility in either sex it can play little or no part in the creation of new species or subspecies, and it is questionable whether chromosomal aberrations of this type have had any significant role in vertebrate evolution. The ambystomid salamanders, with relatively high fertility in both triploid and tetraploid females, probably constitute as favorable material as any vertebrate for the establishment in nature of a tetraploid strain, yet even in this genus the sterility of the male tetraploid and the consequent impossibility of tetraploid matings ($4n \times 4n$) would seem to make the perpetuation of such a strain impossible. From matings of triploid and tetraploid females with *diploid* males, only an "alternation of generations" of polyploid *females* (i.e., $3n$, $4n$, $3n$, $4n$, etc.) such as that described in this paper can provide a continuous line of polyploids,—a line, however, always dependent upon matings with diploid males and the chance production of an occasional tetraploid daughter by the females of the

triploid generations, whose usual progeny (hyper-diploids) are with very few exceptions condemned to an early death by their chromosomal imbalance.

Summary

Triploid axolotl females, or those of mixed axolotl and *Ambystoma tigrinum* ancestry, whether of spontaneous origin or induced experimentally, are of variable fertility, but occasionally may spawn as many as several hundred eggs. Because of the sterility or near-sterility of all polyploid males, however, such females have thus far been successfully mated only with diploids. The resulting offspring, largely hyper-diploid as to chromosome number, are commonly non-viable, but the chance occurrence of a few viable tetraploid females in certain spawnings has permitted obtaining three successive generations of polyploids, — the first triploid, the second tetraploid, and the third again triploid (or near-triploid). Should these third-generation animals in turn produce a few viable tetraploid daughters in addition to the hyper-diploids expected from their matings with diploid males, the "alternation of generations" may be continued beyond the series of three here reported.

The sterility or near-sterility of all polyploid males, the non-viable character of most of the offspring of triploid females mated with diploid males, and, finally, the triploid character of the many viable offspring of tetraploid females mated with diploid males, constitute a combination of factors which would seem to render improbable the establishment in nature of a stable tetraploid strain of ambystomid salamanders. The establishment of such a strain in the laboratory remains a possibility dependent upon obtaining fertile tetraploid males.

Addendum. The third-generation near-triploid female of Figure 3*C* and a comparable third-generation female of white axolotl ancestry have recently spawned, and viable tetraploids of a *fourth* polyploid generation may be found among their several hundred offspring.

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Color and Temperament in Horses

To The Editor:

I have recently read the discussion of coat color, physique and temperament in the September 1947 Journal. As to temperament of many animals, I cannot comment, but as to the relationship of coat color to temperament in horses, I disagree that there is any linkage whatsoever. We have raised horses for 16 years and have had all colors and variety of colors, barring albinos. Not just a few but horses by the hundreds; and knew them all by name and breeding. These were Half-Breds, that is, grade or range mares crossed with Thoroughbred stallions. I could, given time, compile a list containing mares, offsprings, color, disposition and type. But it would still wind up that two full brothers of the same color and conformation would sometimes be very similar in temperament and in other cases extremely different. Chestnuts were as numerous as any other color and ran the gamut from the most tractable, quickest to learn and of great heart to finish a working job, to the kind that would fight, kick, buck, and sulk. There were knot-heads among the grays and bays as well as any other color, and equally desirable tractable ones.

By the way, all of the horses I have mentioned above were broken and handled, and ridden by one man, my husband, so difference in their handling could not have a bearing on the outcome. They were all handled as near alike as could be possible. He comes as close to being a psychologist where the horse mentality is concerned as any one I've ever met. He was raised on a horse ranch and worked with them over 35 years. He has broken wild horses, range and ranch horses and barn raised horses. I, myself, hardly knew a horse from a cow till I married him and moved to the ranch at 22. But I developed an intense interest in them and tried to learn from him as well as by working with horses myself.

Forsythe, Montana Mrs. Francis Sloan

THE EFFECTS OF NITROGEN MUSTARD ON MITOSIS IN ONION ROOT TIPS*

ALVIN NOVICK AND A. H. STERROW†
Biological Laboratories Harvard University

SINCE Muller's[12] discovery of the pronounced genetic effects of x-radiation, an extensive search for other mutagenic agents has been made. It is now known that other ionizing radiations or accelerated particles produce both genetic changes and detectable chromosomal aberrations. However, the search for chemical mutagens of comparable potency had proved disappointing until the discovery by Auerbach and Robson[1] of the mutagenic power of mustard and three other chemically related substances and the recent reports by Strong[14] and Demerec[7] of mutations induced by carcinogens.

In recent papers, the biological actions of *bis* (B-chloroethyl) methyl sulfide and *bis* (B-chloroethyl) methylamine have been reviewed by Gilman and Phillips.[9,10] No specific description of the nature or mechanism of their mutagenic power is given. Auerbach,[2] however, reported mutations in the descendants of treated *Drosophila* and suggested a specific centromere action as the cause of chromosomal rearrangements. In a later review paper, Auerbach, Robson and Carr[3] compared the relative effectiveness of nitrogen mustard and of x-rays in producing lethals and translocations. They suggested that the apparently lower efficiency of nitrogen mustard might be due to an interference with the normal ability of broken ends to rejoin. In this same paper, evidence was cited which indicates that nitrogen mustard may produce mutations which remain latent for several succeeding generations.

McElroy, Cushing and Miller[11] have reported mutations in *Neurospora crassa,* and Stahmann and Stauffer[13] have induced mutations in *Penicillium notatum* through the use of nitrogen mustard. Gillette and Bodenstein[8] have described the striking growth-inhibiting power of nitrogen mustard on the amphibian embryo, *Triturus torosus.* Arrest of growth, followed by degeneration and death, resulted from the immersion of the animals for 45 minutes in a .001% aqueous solution of nitrogen mustard. Histological differentiation, however, continued.

Materials and Methods

The young roots of *Allium cepa* ($2n = 16$) provide favorable experimental material containing abundant mitotic figures readily available for treatment and subsequent fixation. White, yellow and red varieties were used, the yellow proving most satisfactory.

Since some difficulty was experienced in obtaining a sufficient number of *Allium* root tips during the early summer, a preliminary investigation was carried out using the root tips of *Lilium henryi* ($2n = 24$) and of an unidentified species of *Tradescantia* similar to *Tradescantia geniculata* ($2n = 16$).

Healthy onions, partially immersed in jars of tap water, produced fifteen to twenty root tips within five days. Adventitious roots were grown on the sprouts of *Lilium henryi* by enclosing the bulbs in damp jars. Cuttings of *Tradescantia* grown in water developed approximately four roots at each submerged node within two days. All plants were kept at room temperature throughout the experiments.

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†The present addresses of the authors are: Vanderbilt Hall, Harvard Medical School, Boston, Mass., and Brookhaven National Laboratory, Upton, N. Y., respectively.

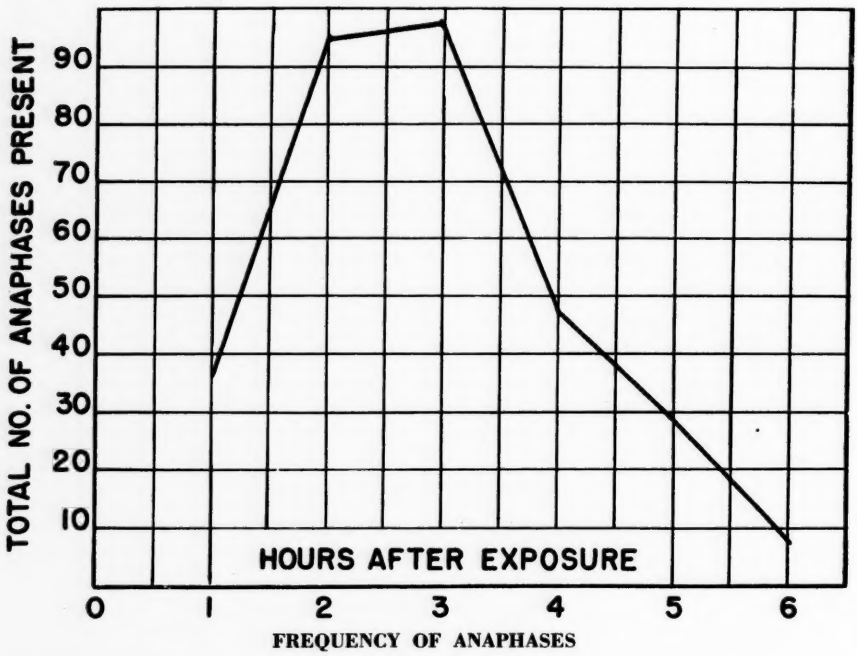

Figure 6

The graph shows the variation in number of anaphases present at various times after treatment with nitrogen mustard. An initial increase is followed, after about three hours, by a rapid decline.

When the experimental plants had grown a sufficient number of root tips, they were transferred to jars containing the freshly prepared nitrogen mustard [*bis* (n-chloroethyl) methylamine hydrochloride] solution for varying periods of time. Care was taken to expose little more than the roots of the plant to the solution and for precautionary purposes, the plants were kept in a closed hood during the exposure period.

After treatment, each plant was washed thoroughly and returned to its original jar to permit further growth to take place. At intervals after treatment root tips were removed and fixed in 3:1 alcohol acetic, hydrolyzed in 1:1 concentrated HCl and 95% ethyl alcohol, stained with aceto- or propiono carmine, and squashed.

Control roots were removed from each plant and examined for aberrations immediately before treatment, and untreated control plants were examined periodically during the post-exposure interval.

Since nitrogen mustard reacts with water and many impurities, as well as with cell constituents, it is important to stress that, undoubtedly, there were changes of concentration of nitrogen mustard in the solutions used, especially during long exposures. Gillette and Bodenstein[8] state, however, that the decomposition rate in water is relatively slow.

Observations

The root tips were studied cytologically in order to determine whether any mitotic inhibition or chromosomal aberrations had been induced. The effects observed will be described below under the headings of physiological and cytological effects, although the two are probably interrelated.

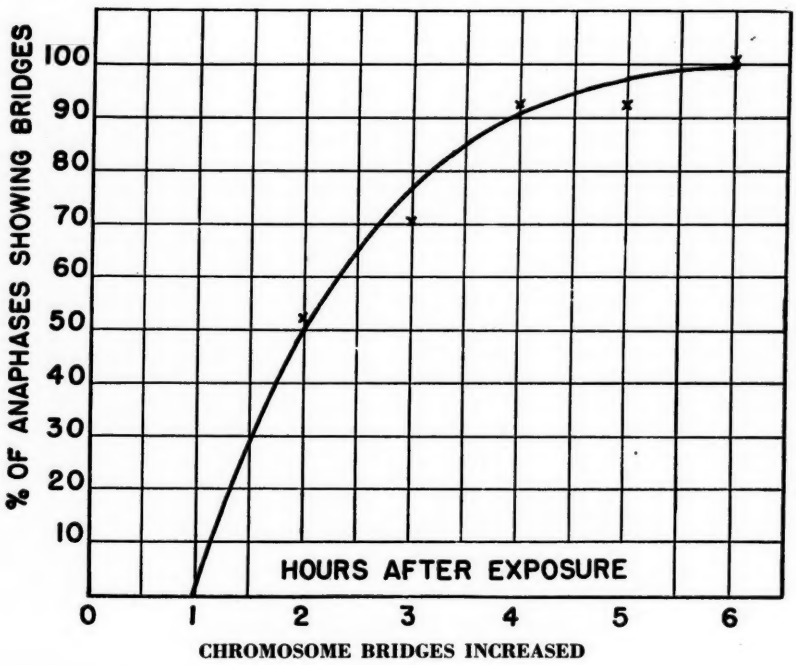

Figure 7

Graph shows gradual increase in percentage of anaphases showing sticky chromosome bridges up to six hours after treatment.

Physiological Effects

In all the plants exposed to nitrogen mustard, mitoses disappeared in about six hours. This was followed by an arrest of growth for a period varying from approximately 20 hours to a permanent condition of stasis, depending upon the dosage received. In the more lightly treated plants, a period of aberrant divisions followed this period of inhibition. Permanent cessation of growth frequently followed the period of aberrant mitoses. In those plants which showed few aberrations, however, growth returned to normal within a few days.

In *Tradescantia* and *Lilium,* divisions disappeared within three and four hours respectively after exposure. In *Allium,* at a concentration of .005%, divisions became progressively fewer after two hours and had almost completely disappeared by six hours (see Table I and Figures 6 and 7).

Growth stopped more quickly after higher doses of nitrogen mustard. During this period of inhibited growth which followed, differentiation continued, but no complete mitoses occurred. Cells in prophase were frequently observed, but apparently failed to complete the division. In those plants which resumed growth after the period of inhibition, a normal number of mitoses continued to occur for three or four days. The roots of some of these plants then ceased all growth, while other plants, apparently more resistant, continued to grow. The longest period of inhibition, from which a plant was observed to recover, was over 96 hours.

Cytological Effects

A survey was made of all the anaphases present on each slide, and the percentage with chromosome bridges and/or fragments was calculated. Other cyto-

logical aberrations were observed in treated root tips while examination of untreated controls revealed no aberrations.

The cytological picture observed at hourly intervals during the first six hours following exposure of *Allium* to 0.005% solution of nitrogen mustard is illustrated graphically in Figures 6 and 7. At the end of the first hour divisions were abundant and completely normal (see also Table I). After two hours from 1 to 16 sticky bridges were evident in 52.5% of the anaphases. The percentage of such aberrant anaphases gradually increased with time to 100% at six hours. After the third hour the total number of anaphases fell off rapidly. At 12 and 24 hours after treatment with 0.001% solution, no divisions were present (Table II). Plants that recovered from the inhibition all showed abnormal mitotic figures characterized by true chromatin bridges and fragmentation. The percentage of aberrant cells reached a maximum of 75% at 96 hours and decreased to 47% by 144 hours (Table II).

In certain experiments, the plants never recovered from the initial inhibition. In these cases the concentrations used were 0.001% for 20 minutes for *Tradescantia*, 0.002% for 30 minutes for *Lilium*, and 0.01% for 30 minutes for *Allium*. A somewhat lower dosage of 0.0005% in *Lilium* and *Tradescantia* for 10 and 15 minutes respectively, had practically no effect. Mitotic inhibition occurred, but very few chromosomal aberrations were observed. The minimum effective dosage for onions was found to be 0.001% solution for 30 minutes. The root tips showed abnormal divisions from the second to the sixth day after treatment. The presence of both bridges and fragments in a single cell was very common, but cells with bridges only were also frequently observed. In approximately 20% of the cells, two or more bridges appeared together.

In addition to these aberrations, tetraploidy and chromosomal disintegration during both metaphase and anaphase were occasionally noted. In some cells, the chromosomes were abnormally elongate, beady, and lightly stained.

Unfortunately, the data obtained did not indicate any relationship between dosage and percent aberration. The variation seems more likely to be attributable to differences in individual plant susceptibilities. Further, there was no clear correlation between duration of growth inhibition and percent aberration.

TABLE I. Anaphase sticky bridges induced in *Allium* after exposure to 0.005% solution of nitrogen mustard in distilled water for 30 minutes.

Hours after treatment	Total no. of anaphases	Percentage with sticky bridges
1	37	0
2	95	52.5
3	98	70.5
4	47	92
5	29	93
6	8	100

TABLE II. Anaphase aberrations induced in root tips of *Allium* at various times after exposure to 0.001% solution of nitrogen mustard in distilled water for 30 minutes.

Hours after treatment	Total no. of anaphases	Percentage aberrant
12	0	–
24	0	–
48	90	49
96	67+	75
144	149	47

Discussion

The inhibiting effect of nitrogen mustard on mitosis in *Allium* root tips is conclusively shown. Even the smallest dose used was sufficient to cause an inhibition lasting for almost 24 hours. Larger doses stopped growth permanently. After a period of aberrant mitoses a second cessation of growth occurred. It can probably be attributed to the death or lack of vigor of daughter cells suffering from loss of vital chromatin material.

Divisions present at the time of exposure can probably be divided into two groups on the basis of their subsequent behavior—those in early prophase and those in later stages. Cells approaching or past metaphase probably complete division normally. Those treated at late prophase have a low sensitivity, while those treated in earlier prophase presumably have a higher sensitivity since the percentage showing sticky bridges increases up to six hours.

Since prophases were present in all root tips even during the period of inhibition, it seems likely that the effect of the

nitrogen mustard occurs mainly during prophase. The occasional total absence or great scarcity of prophases in heavily treated plants suggests that an inhibitory action may also occur during the late resting stage or may cause a reversal to resting stage similar to that reported by Carlson[5] following exposure to irradiation. The mechanism may, however, be destructive rather than inhibitory. The threshold concentration needed to arrest division in the resting stage is apparently higher than that for inhibitory action in prophase.

The period between exposure and the reappearance of mitosis in the roots is related to the dosage. Divisions disappeared more quickly after heavy treatment than after treatment with low concentrations. Individual response varied considerably more than the variation with dose.

The mechanism by which the bridges and fragments are induced is still obscure, although it is known that the mustards have an affinity for nucleoproteins and may cause intranuclear precipitation.[4] Darlington and Koller[6] attribute the effects to injuries to the nucleic acid metabolism and the protein structure.

The diminution in number of bridges with increased dosage may be traced in part to the possibility predicted by Auerbach for the mustards and nitrogen mustards. Since fragments are frequently too small to be detected readily and are easily obscured by chromosome masses, it seems likely that more aberrations occurred than were recorded.

It is suggested that the threshold concentration for chromosomal aberrations is higher than the inhibition threshold, since several plants showed inhibition but not aberrations.

In addition to variation in individual susceptibility, the three plant genera differed greatly in their reactions. The concentration necessary to cause aberrations in *Lilium* and *Tradescantia* is very close to the concentration which permanently stunts their growth. In red and yellow onions, however, this range is relatively great. White onions showed high resistance to both mitotic inhibition and to the production of aberrations, possibly because of their larger and thicker roots. It is important to stress that the general vigor of the plant was usually not permanently impaired by the lower doses of nitrogen mustard. All but the most heavily treated plants eventually developed new, normal roots.

Summary

Growing root tips of *Allium cepa, Lilium henryi* and *Tradescantia* sp. were treated with nitrogen mustard in aqueous solution at concentrations ranging from .0005% to .01% and for varying periods of time. In the cases studied, mitoses were usually eliminated within six hours. Then followed a period of mitotic inhibition during which no cell divisions were observed although differentiation continued. This period varied from 20 hours at low dosages to over four days at high dosages. After heavy treatment (.01% for 30 minutes in *Allium*) recovery was not observed in the treated root tips.

In those plants which received a smaller dosage (.001% for 30 min.) marked chromosomal disturbances were observed as described in the text.

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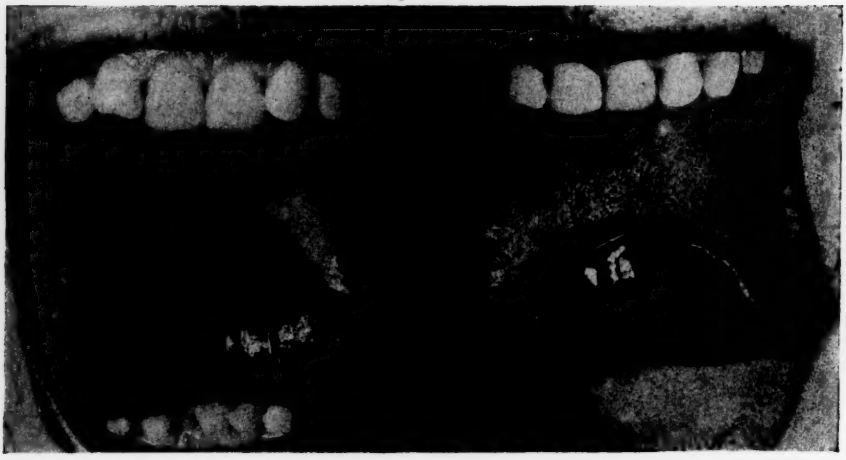
TONGUE TIP OVERFOLDING*



TWO WAYS TO FOLD A TONGUE

Figure 8

Overfolded tip of the tongue, inside and outside of the mouth, of a young white man. About 1½ per cent of 1,000 students could overfold the tongue-tip within the mouth. Only two men and one woman—among about 1,800 students could overfold the tip of the extended tongue.

THE tongue is a very mobile organ capable of a variety of intricate movements. However, some persons are able to perform certain types of tongue movements which other persons are unable to. One of these movements is the folding back of the tip of the tongue as shown in Figure 8.

Among about 200 students in a course in genetics only four were found who could fold back the tip of their tongues; and from another group of about 800 students in the various courses in zoology ten more were found who could do this. Twelve of these had often done this as children. The other two, although successful on the first trial, had never before heard of such ability. Ten of these 14 students were boys.

In eight of the fourteen families represented, neither parent could fold back the tongue. In one family the mother was a "folder." For the remaining five families information was not available. One of the eight students whose parents are negative has a sister who can fold the tip of her tongue. This sister has a small son similarly gifted.

Some other traits were tabulated to find out whether any of them were associated with this tongue-folding trait. These traits were the well-known lateral edge-curling or rolling of the tongue which was found to be a dominant trait by Sturtevant; twisting the tongue so that the laterial sides would be dorsally and ventrally placed; extending the tongue so as to touch the tip of the nose; and swallowing the tongue. Only six of the fourteen persons could perform all of these tongue maneuvers and one was negative for lateral edge rolling. No correlation with other traits was noted.

The present study in a white population seems to corroborate the findings of Hsu[1] that among Chinese overfolding is recessive.

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David D. Whitney
University of Nebraska

*Studies from the Department of Zoology, University of Nebraska, No. 239, with aid of grant from the University of Nebraska Research Council.

TONGUE-FOLDING AND TONGUE-ROLLING

In a Sample of the Chinese Population

T. T. Liu and T. C. Hsu
Department of Biology, National University of Chekiang, Hangchow, China

Sturtevant[4] found that there exist two fairly distinct classes with respect to the ability to turn up the lateral edges of the tongue. In his opinion this ability is possibly due to a single dominant gene. Recently, Urbanowski and Wilson[5] also reached the same conclusion. Apart from the rolling up of the tongue edges, another character of tongue movement, the upfolding of the tongue tip, has been noted by Hsu[1] and reported in a separate paper. According to his finding, this character is certainly caused by a single Mendelian factor, the individuals with the ability of tongue-upfolding being homozygous for the recessive gene. The purpose of this paper is to report the results of population studies of these two characters in populations and to investigate the relation between these two pairs of genes.

Material

The survey was carried out at Chekiang University and the University Middle School, Hangchow, China. The abilities of tongue-folding and tongue-rolling were tested simultaneously. The subjects examined at Chekiang University were freshmen whilst in the University Middle School, all the students were included. The data here collected inevitably involve some errors in regard to the ability of tongue-folding. One source of error is the psychological effect, since these tests were made in the course of physical examinations wherein the students' fear of discovery of physical defects may have affected the tongue rolling and folding abilities. The other difficulty is in the tongue-folding trick itself. As reported by Hsu, though the majority of the persons who possess the ability of tongue-folding can do this trick instantly without previous practice, in a few persons it is acquired only by practice. During the course of testing, the latter are erroneously included in the inability class. Naturally these errors may, more or less, obscure the data, but the effect seems very slight and may be neglected.

Results

Table I shows the total data we obtained. The sexes are recorded separately and the subjects are also classified according to geographical origin. In this table, the symbol *r* represents the tongue-rolling ability, and *f*, the tongue-folding ability. The plus sign (+) indicates the persons who can do the respective trick and the minus sign (−), the persons who cannot do that trick. (We will use these same symbols throughout the paper.)

Table II shows that although the proportion of persons who can roll but not fold the tongue is similar in the two sexes, there is a sex difference in the ability to both roll and fold the tongue in favor of women.

From Tables I and II one may note the interesting fact that no people who can fold up but not roll the tongue have been discovered. Whether this absence is due to chance or other cause needs further study. If *A–a* is a pair of genes responsible for tongue rolling, the genotype of people who can roll is either *AA* or *Aa*, and of people who cannot do it is *aa*. If *B–b* is another pair of genes responsible for the tongue folding, the people who possess this ability are *bb*, while the people incapable of doing it are either *BB* or *Bb*. Then the genotype *A–bb* can do both the tricks. *A–B–* can

roll the tongue, *aabb* can fold up the tongue, and *aaB–* cannot do either. Now, assuming a random combination of these two pairs of genes, we should obtain the results as shown in Table III.

From Table III, the expected number in class *aabb*, that is, the people who can fold up but not roll the tongue, is 12.85, but the observed number is zero, χ^2 is 21.35, and the probability is less than 0.01. Thus it is obvious that the absence of the *aabb* class is not due to chance, but is caused by other factors.

The linkage between these two pairs of genes might be the cause. But Hsu and Liu[2] have pointed out that in a random sample of a population, when the equilibrium state has been reached, neither linkage nor independent assortment would interfere with the random combination of the related genes.

In view of this fact the authors must offer a new explanation. Assuming that *A* and *B* are complementary, the expression of *b* must depend on the presence of *A*, otherwise the *b* effect cannot be expressed.

If this is the case, the first thing we could do is to find out the proportion of persons who are homozygous for the recessive tongue folding gene among the phenotypical negatives, and compare the expected number with the observed numbers so as to see whether the explanation is probable.

If x is the proportion of persons homozygous for *aabb* among the phenotypical negatives, then

$$34:615 = x:(394-x), \text{ and}$$
$$x = 20.64$$

This means that among the phenotypical negatives, 20.64 individuals should have the genotype *aabb*. From the above calculations we could obtain the frequencies of the two tongue-movement characters separately (Table IV).

Since the interaction of the two pairs of genes is clear, then the gene frequencies both for tongue-rolling and tongue-folding can be obtained easily (Table V). The frequency of *A* is 0.39. and of *b* is 0.23.

Discussion and Conclusions

Examples of complementary factors in animals and plants are not rare, such as flower color in the common yellow daisy and coat colors in rodents. In human

TABLE I. Geographical distribution of tongue rolling and tongue upfolding characters.

Province	Can Roll and Fold r+ f+ Actual No. ♀	♂	%	Can Roll but Not Fold r+ f– Actual No. ♀	♂	%	Can Neither Roll nor Fold* r– f– Actual No. ♀	♂	%	Total ♀	♂	Grand Total
Anwhei	0	0	0.00	12	17	60.42	9	10	39.58	21	27	48
Chekiang	8	8	2.77	82	255	58.41	36	188	38.82	126	451	577
Kiangsu	1	3	2.41	36	62	59.04	16	48	38.55	53	113	166
Other provinces (16)	9	5	5.56	70	81	59.92	35	52	34.52	114	138	252
Total:	18	16	3.26	200	415	58.96	96	298	37.78	314	729	1,043

*No persons who could fold but not roll the tongue were found.

TABLE II. Frequency of two tongue movement characters in relation to sex.

Sex	Can Roll and Fold r+ f+ Actual No.	%	Can Roll but Not Fold r+ f– Actual No.	%	Can Neither Roll nof Fold r– f– Actual No.	%	Total Actual No.	%
♀	18	5.73	200	63.70	96	30.57	314	100
♂	16	2.19	415	56.93	298	40.88	729	100
Total	34	3.26	615	58.96	394	37.78	1,043	100

TABLE III. Test for independence of two tongue movement characters.

Genotypes	*A–B–*	*A–bb*	*aaB–*	*aabb*
Phenotypes	r+ f–	r+ f+	r– f–	r– f+
Observed	615	34	394	0
Expected	627.80	21.15	381.2	12.85

$\chi^2 = 21.35$, $P < 0.01$

TABLE IV. Proportions of tongue rolling and tongue folding characters.

	Rolling		Folding			
Phenotype	+	–	+		–	
Genotype	*AA+Aa*	*aa*	*bb*		*BB+Bb*	
Actual no.	649	394	34.00 / 20.64	54.64	615.00 / 373.36	988.36
%	62.22	37.78	5.24		94.76	

TABLE V. Gene frequencies.

Rolling		Folding	
A	*a*	*B*	*b*
	$\sqrt{.3778}$		$\sqrt{.0524}$
0.39	0.61	0.77	0.23

beings only one possible case has been reported by Snyder[3] where deaf-mutism appears to depend on one combination of two factors.

The tongue genes *A* and *b* act on different characters, and the former gene can produce the character in heterozygous condition, while the latter gene can produce the character only in the homozygous recessive condition. Furthermore, the development of the folding character depends on the presence of the dominant condition *A*.

As to the cause of the relationship between these two characters, it is probably due to the structure of the tongue muscle. Folding up the tongue tip is a rather delicate trick which requires the movement of many muscles simultaneously. The rolling process is simpler, as compared with the folding process. In those persons who can fold up the tongue tip, the structure of the tongue muscle can also meet the requirements of the rolling process.

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HERITABLE CHARACTERS IN MAIZE

Linkage of a Factor for Shrunken Endosperm with the a_1 Factor for Aleurone Color*

E. B. Mains
University of Michigan, Ann Arbor, Michigan

In 1942, an open-pollinated plant of maize having colorless aleurones and liguled leaves was crossed with a plant of an inbred line (*LA*) having colorless aleurones and liguleless leaves for the purpose of obtaining material to demonstrate the inheritance of liguleless. The pistillate parent was from commercial seed of unknown ancestry. The staminate parent was from seed which was 12 years old. The germination was poor but enough plants were obtained for the purposes of pollination. In addition to being homozygous for liguleless, line *LA* carried the factors for aleurone color, *aaCCRRPrPr*. The ear resulting from the cross had purple kernels indicating that the pistillate parent carried aleurone factors *AA*. Results obtained in following generations indicate that the pistillate parent had *pr* and either *c* or *r* or probably both as homozygous recessives.

In 1943, 19 self-pollinated ears were obtained which showed multi-factor segregation for aleurone color. Among the kernels with colorless aleurones were a number which were shrunken. No colored† kernel showed the shrunken condition. Hutchison[2,3] and others have shown that a factor for shrunken endosperm (designated *sh*) is closely linked with the factor *c* for aleurone color. As can be seen by comparing Figure 9*A-C* with Hutchison's figures, there is considerable resemblance between his "shrunken" and ours. It was assumed that the factors involved in the above cross were

*Paper from the Herbarium and the Botany Department of the University of Michigan. Facilities for growing the lines of maize were furnished by the Botanical Gardens. The writer is indebted to L. F. Randolph for supplying tester lines for use in this study and to M. M. Rhoades for reading and criticizing the manuscript.

†Since *Pr* only modifies the expression of color and is not important in this study, red and purple aleurones are classified together as colored.

sh and *c* derived from the pistillate parent. At first an attempt was made merely to obtain material to demonstrate linkage for teaching purposes, but we encountered an anomalous situation in terms of the above assumption. In 1944 six ears resulting from test-crosses of plants grown from colored full kernels with plants from kernels having shrunken endosperms and colorless aleurones, gave ratios approaching 1 colored to 1 colorless aleurones and showed a close linkage of shrunken endosperm with colorless aleurone. There were only 5 crossovers in a total of 1,316 kernels, giving a crossingover percentage of only 0.38. This is a considerably closer linkage than had been reported for *c* and *sh* for which Emerson, Beadle and Fraser[1] in their summary of linkage studies give percentages varying from 3.0 to 5.8 with an average of 3.3. We designated our lines segregating for shrunken as "*SH*-lines."

In 1945, among the selfs of plants from full kernels with colored aleurones, five ears (*SH*-lines) were obtained which showed segregations closely approaching 3 colored to 1 colorless, with shrunken closely linked with colorless and therefore indicating a segregation only for the aleurone color factor linked with shrunken. Later results proved that this was correct. The ratio for the five ears was 1,132 colored, full kernels; 2 colored, shrunken; 1 colorless, full; 394 colorless, shrunken. The ratio of 1,134 seeds with colored aleurones to 395 with colorless is close to that expected for one heterozygous pair of factors (Dev. 12.75 and P. E. 11.42). Likewise the ratio of 1,133 full kernels to 396 shrunken indicates that a single factor is concerned in the inheritance of shrunken. This is substantiated by the results from 29 selfed ears from heterozygous plants in the course of the study which gave 7,081 full kernels and 2,321 shrunken. The theoretical ratio for a single pair of factors would be 7,051.5:2,350.5 (Dev. 29.5 and P. E. 28.32). The results obtained throughout the study from backcrosses with the double recessive also support this conclusion, where the ratio 11,942 full kernels and 11,976 shrunken is very close to the theoretical 1:1 ratio (Dev. 17 and P. E. 52.16).

In 1945, a number of crosses were made between heterozygous plants from full kernels with colored aleurones and plants from shrunken kernels with colorless aleurones. The results from those crosses involving only the aleurone factor linked with shrunken are given in Table 1. Only 28 crossovers were obtained in 11,852 seeds giving a percentage of crossing-over of only 0.24. Figure 9*B* illustrates the close linkage of shrunken and colorless aleurone and the occurrence of two colorless-full crossovers. Figure 9*C* shows one colored-shrunken crossover.

In 1946 and 1947 the study was continued with the *SH* lines which were segregating only for the factor for aleurone color linked with shrunken. As shown in Table I, the percentage of crossing-over was 0.25 in 1946, 0.39 in 1947 and 0.30 for the two years com-

TABLE I. Linkage of the aleurone factors for color-colorless A_1a_1 and for full-shrunken endosperm Sh_2sh_2 as shown by the cross $A_1Sh_2/a\ sh_2 \times a_1sh_2/a_1sh_2$.

Pedigree number	—Colored— Full	Shr.	—Colorless— Full	Shr.	Total	Cross-overs	C.O.%
1946							
SH11×12–1	157	0	0	155	312	0	0.00
–2	110	0	0	114	224	0	0.00
–3	241	0	1	222	464	1	0.22
–4	137	0	1	112	250	1	0.40
SH12×11–1	138	0	0	127	265	0	0.00
–2	153	0	1	145	299	1	0.37
–3	186	1	2	201	390	3	0.77
–4	186	1	0	168	355	1	0.28
–5	151	1	0	164	316	1	0.32
–6	163	0	0	145	308	0	0.00
–7	90	0	0	89	179	0	0.00
–8	115	1	1	129	246	2	0.81
–9	159	0	0	153	312	0	0.00
–10	197	0	0	169	366	0	0.00
–11	130	0	0	129	259	0	0.00
–12	136	0	1	135	272	1	0.37
–13	119	0	1	111	231	1	0.43
–14	128	0	0	126	254	0	0.00
–15	176	0	1	179	356	1	0.28
SH13×14–1	97	0	0	74	171	0	0.00
SH14×13–1	173	1	0	192	366	1	0.27
–2	220	0	0	224	444	0	0.00
–4	166	1	2	148	317	3	0.95
–5	120	0	1	96	217	1	0.46
Subtotal	3648	6	12	3507	7173	18	0.25
1947							
SH1×6	146	0	0	171	317	0	0.00
SH1×7	185	0	0	156	341	0	0.00
SH1×8	313	1	1	315	630	2	0.32
SH1×9	179	2	2	210	393	4	1.02
SH2×6	312	1	0	302	615	1	0.16
SH2×7	183	0	1	175	359	1	0.28
SH2×8	135	4	1	188	328	5	1.52
SH2×9	307	1	0	286	594	1	0.17
Subtotal	1760	9	5	1803	3577	14	0.39
Total	5408	15	17	5310	10750	32	0.30

CLOSE LINKAGE BETWEEN ALEURONE COLOR AND SHRUNKEN ENDOSPERM

Figure 9

A—Enlarged (1.5×) portion of ear showing kernels with shrunken endosperms and colorless aleurones, a_1sh_2/a_1sh_2; *B*—Portion of an ear of maize resulting from the cross $A_1Sh_2/a_1sh_2 \times a_1sh_2/a_1sh_2$ showing the associated inheritance of shrunken endosperm and colorless aleurone and the occurrence of two crossovers, full endosperm and colorless aleurone, near the middle of the ear; *C*—A crossover similar to that shown in *B*, with a single shrunken-colored crossover near the middle of the ear.

bined. For the entire study 65 crossovers were obtained in 23,918 kernels giving a percentage of crossing-over of 0.27.

The very close linkages between shrunken and colorless aleurone obtained in the *SH* lines raised considerable doubt concerning the identity of the factor for aleurone color. To settle this question a line of maize carrying the *c sh* factors was obtained from L. F. Randolph to whom the writer is greatly indebted for supplying this and other lines. This line had the aleurone factors *AAccRR*. Five crosses were made between it and our homozygous *SH* lines for shrunken and colorless aleurone. All the kernels of the resulting ears had full endosperms with colored aleurones. From this it is evident that the recessive factor of our *SH* lines is not *c*. The line received from Randolph showed considerably less shrinkage of the endosperm than described by Hutchison. This was also true for *csh* material received from G. F. Sprague which was not used in this study. Randolph informs me that there is considerable variation in the expression of the *sh* linked with *c*.

Two crosses were also made with a

line of maize having colorless aleurones and the factors *AACCrr*. Both ears had kernels with colored aleurones indicating that the recessive aleurone factor of the *SH* lines is not *r*.

To test the identity with factor *a*, eight crosses were made with a line (LA) having colorless aleurones and the factors a_1a_1CCRR. All produced seed with colorless aleurones. Also seven crosses were made with a line received from L. F. Randolph which has the aleurone factors a_1a_1CCRR. Likewise they all produced kernels with colorless aleurones. From these results it is concluded that the factor for shrunken endosperm of our *SH* lines is linked with the a_1 factor for aleurone color. It is therefore distinct from the shrunken reported by Hutchison and is designated as *shrunken-2* (sh_2). Its linkage with a_1 places it on chromosome 3.

As can be seen from Figure 9, the shrunken endosperm produced by *sh* is very pronounced and there is no difficulty in the classification of it. The shrinking of the kernel may show at the top as a deep indentation which may be a crease or a broad cup, Figure 9*A*. It also shows as shrunken areas on the sides of the kernels, sometimes without much evidence of the shrinkage when seen from above. The shrunken kernels often have cavities in the upper part as described by Hutchison. The germination of shrunken seeds of the *SH* lines is fairly good under favorable conditions for development.

Summary

A factor for shrunken endosperm (sh_2) in maize has been found to be very closely linked with the factor a_1 for aleurone color giving crossing-over percentages of 0 to 1.52 with an average of 0.27.

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THE PHYTO-CHEMICAL REVOLUTION

ALL parts of this book* will be of interest to plant breeders who will appreciate an authoritative up-to-date readable book on chemicals in the control of plant growth.

The authors picture a chemical revolution sweeping through the agricultural world unrivaled by any of the previous great advances in agriculture. "For the first time man can change the pattern of growth and development of plants, can retard growth here or speed it there. The growth-controlling hormones and other chemicals now in use are but crude beginnings." Chemicals in relation to the rooting of cuttings, blossoming thinning and harvest drop of fruits, fruit set, seed treatment, weed control and breaking the rest period or prolonging it and in the production of new varieties are discussed. For the use of the plantsman, a table of 71 pages summarizes the information on the use of chemicals in the rooting of different kinds of plants. The authors have reviewed and digested the extensive literature, and presented the results in a well-organized book.

Very few corrections are noted. Fig. 5 on p. 210 is misleading. Sectors in angiosperm stems are not so simple as that illustrated. Rather most polyploid sectors of stems are at first mericlinal. When shoot development occurs from such sectors, they become periclinal.

Geo. M. Darrow

*Hormones and Horticulture by George S. Avery, Jr., and Elizabeth B. Johnson published by McGraw-Hill Book Company, New York City, 326 pp., $4.50.

MEIOSIS IN COLCHICINE INDUCED TETRAPLOIDS OF RHOEO

DALE C. BRAUNGART
Department of Biology, Catholic University of America
Washington, D. C.

SPONTANEOUS reciprocal translocations have rearranged the diploid chromosome complement of *Rhoeo discolor* Hance to the extent that no individual chromosome has a structurally identical homologue. Terminalization of chiasmata between homologous chromosome segments results in the formation of rings and chains at metaphase of the first meiotic division.[3,6,7] Since each chromosome in diploid *Rhoeo* is structurally unique, the possibility for the chromosome complement to pair as bivalent homolgous chromosomes exists only in the tetraploid where each chromosome would have a structurally identical homologue. Although homologous chromosomes are present in duplicate in tetraploid *Rhoeo,* homologous chromosome segments are present in quadruplicate. If chiasmata formation were restricted to completely homologous chromosomes, twelve bivalents would be observed at metaphase I of the tetraploid. However, if chiasmata formed between homologous chromosome segments, a variety of configurations including rings and chains would be anticipated at first meiotic metaphase.

Haselwarter[5] induced tetraploidy in individual microsporocytes of *Rhoeo* by temperature shock, and Dermen[4] produced tetraploid cells with colchicine. Akemine[1] also observed tetraploid microsporocytes along with the usual diploids after subjecting *Rhoeo* to low temperature. They all reported a variety of configurations including univalents, rings and chains. Akemine reported ring bivalents but the organization of the complement into 12 bivalents at metaphase I was not observed by any of the above investigators.

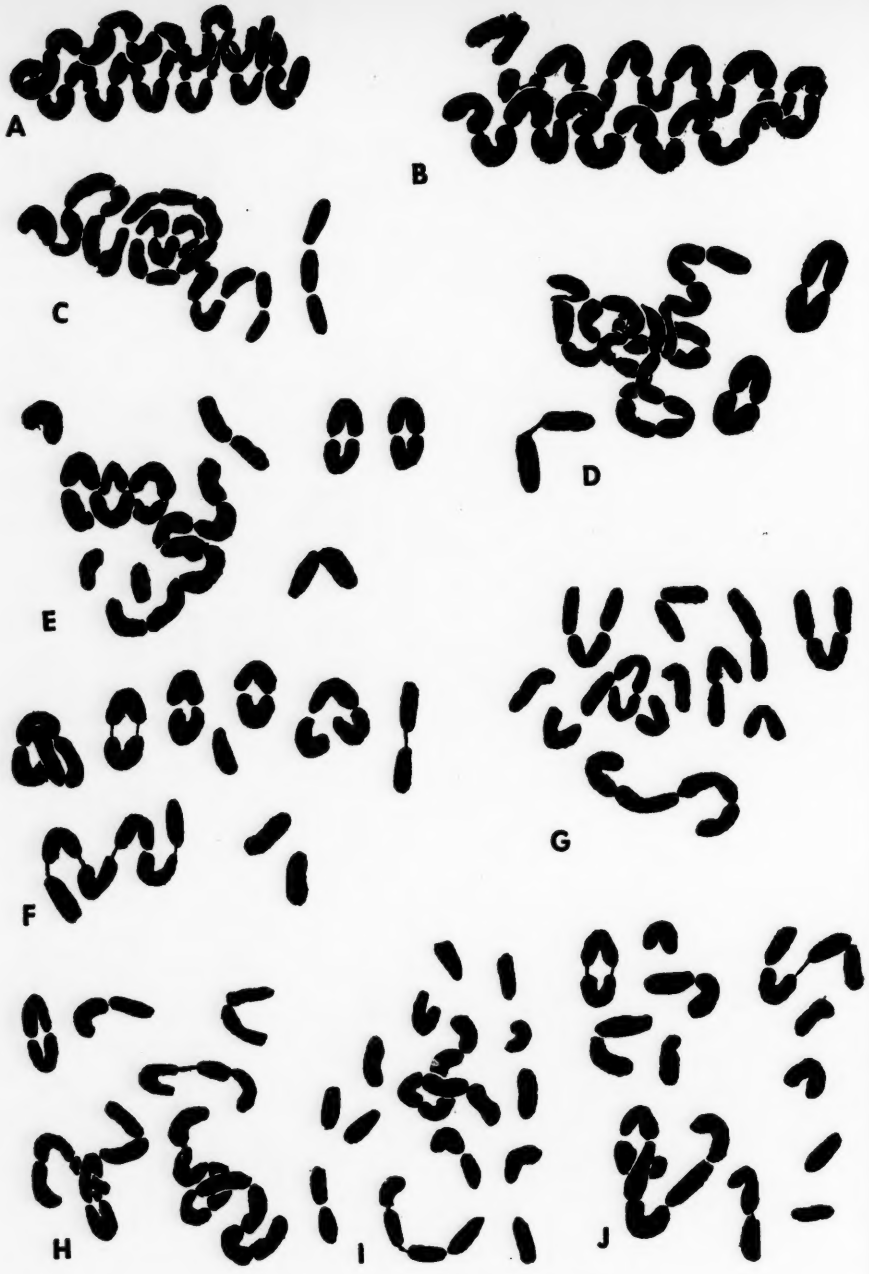
The observations of Haselwarter, Dermen and Akemine were based on individual tetraploid cells arising from the immediate effect of polyploidy inducing treatment of diploid plants. The present study of meiosis in colchicine induced tetraploid plants was initiated to compare meiotic configurations in the tetraploid plants with those observed in individually treated cells. While the present study was in progress, Walters and Gerstel[7] observed and reported on meiosis in a spontaneous tetraploid of *Rhoeo.* In general their results are in accord with the observations on individually treated cells.

Procedure

Six apparently tetraploid plants from colchicine treated seedlings were obtained from Dr. Haig Dermen of the Plant Industry Station at Beltsville, Maryland, in 1941. All six plants were taller and more robust than the diploids. Propiono-carmine smears were utilized to obtain chromosome counts of the root tips of these plants and to study meiosis in the induced tetraploids. Four plants were aneuploid, having 22 chromosomes; and two plants had the tetraploid number of 24. The aneuploid plants did not flower.

Results

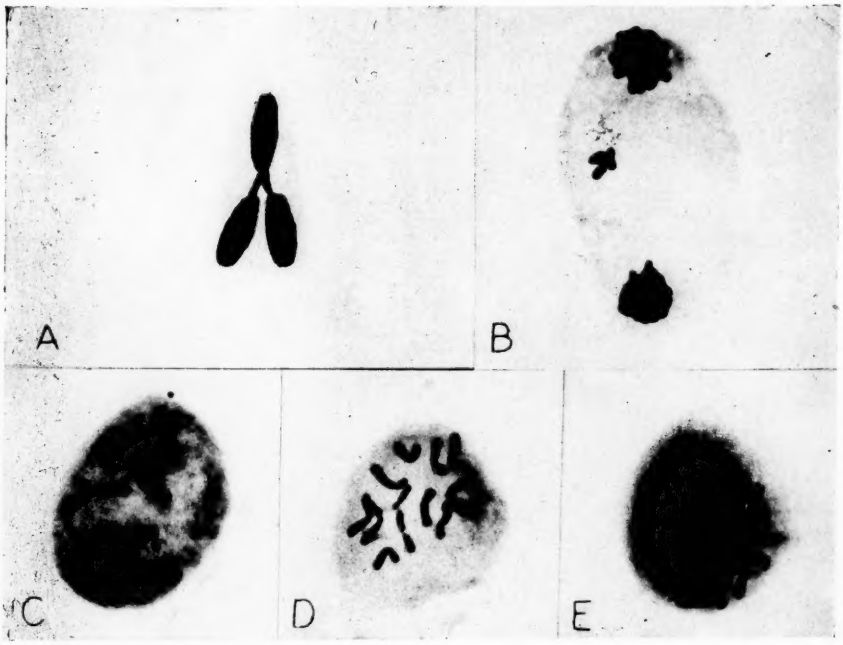
The meiotic configurations of 202 microsporocytes were studied. The results of these observations are recorded in Table I. It was possible to determine the exact association of all 24 chromosomes in only ten of the cells reported in Table I. A detailed account of these ten cells is given in Table II and Figure 10. Short chains were the most frequent type of configuration encountered, with chains of two chromosomes most numerous. The next most frequent association was pairs of chromosomes which were found in approximately one-half of the



RHEO CHROMOSOME CATENATIONS

Figure 10

Camera lucida drawings of chromosome arrangements found in meiotic cells in tetraploid Rhoeo. *A*—Chain of 24 chromosomes; *B*—Chain of 22 chromosomes and one pair; *C*—Chains of 21 and three chromosomes; *D*—Chain of 18 chromosomes and three pairs; *E*—A ring of six chromosomes, a chain of seven, two chains of two, two pairs, and three singles; *F*—Ring of four, ring of three, chain of six, chain of two, three pairs and three singles. Other arrangements are shown in figures *G, H, I,* and *J*.



MEIOTIC IRREGULARITIES

Figure 11

A—*Y*-configuration of three chromosomes associated by two chiasmata; *B*—Nucleus containing a lagging chromosome at telophase I; *C*—Microspore with 11 chromosomes; *D*—microspore with 12 chromosomes; *E*—microspore with 13 chromosomes.

cells. Three pairs per cell was the maximum number observed. Closed rings of 3-6 chromosomes occurred infrequently in comparison with chains. Of the ring configurations, those involving four chromosomes were most numerous. Three chains of 24 chromosmes were observed but a complete and closed ring of 24 was not found. Varying numbers of univalents were found in 85 of the 202 cells observed. Y-shaped configurations of three chromosomes associated by two chiasmata (Figure 11*A*) were observed in twelve cells.

Meiosis in the induced tetraploid resulted in irregular chromosome numbers in the pollen. Analysis of meiotic telophase configurations disclosed frequent lagging of chromosomes. Microspores having 11, 12, and 13 chromosomes are illustrated in Figure 11.

Discussion

The absence of meiotic prophases fa-

TABLE I. Metaphase configurations observed in 202 cells.

Type of configurations	Pairs	CHAINS																							RINGS				Univalents	2 Chiasmata (one segment)
		2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	3	4	5	6		
Total of each configuration	135	173	81	50	26	15	17	7	2	3	1	5	1	2	0	1	0	1	1	0	1	2	0	3	1	13	2	1	274	13
Number of cells exhibiting each configuration	91	102	63	41	23	13	16	7	2	3	1	4	1	2	0	1	0	1	1	0	1	2	0	3	1	6	2	1	85	12

vorable for detailed analysis along with localization and terminalization of chiasmata in *Rhoeo* excluded the possibility of determining whether complete pairing occurred between homologous chromosomes in the induced tetraploid. However, the presence of chromosome pairs in which the two chromosomes have terminal chiasmata at both ends (Figure 10*D, E,* and *F*) indicates pairing between homologous chromosomes at least in subterminal regions. That ring and chain configurations were relatively more frequent than chromosome pairs indicates that homologous segments of non-sister chromosomes are paired more frequently than completely homologous chromosomes.

In general the meiotic configurations of the induced tetraploid paralleled those of the spontaneous tetraploid studied by Walters and Gerstel. Lagging chromosomes, frequent univalents and micronuclei in the tetrads were observed in both studies. Rings of eight reported by Walters and Gerstel in the spontaneous tetraploid were not observed in the present study; and rings of three and five found in the induced tetraploid were not reported by Walters and Gerstel.

Interstitial chiasmata were not found in the induced tetraploid, bearing out the observations of Bhaduri,[2] although Walters and Gerstel clearly illustrate an interstitial chiasma in their analysis of the spontaneous tetraploid. Complete terminalization of chiasmata was observed in the present study, since all chromosome associations were of the end-to-end type.

TABLE II. An analysis of the metaphase configurations of Figure I.

		Chains of										Rings of	
Section	Pairs	2	3	4	6	7	8	18	20	21	22	24	6	Univalents
A												1		
B			1								1			
C				1						1				
D	2		1					1						
E	2		2			1							1	3
F	3		1	1	1	1								3
G			3	3	1									5
H	2		3	2				1						
I			2		1	1								10
J	1		3		1	1								6

Akemine observed triple terminal chiasmata in individually induced tetraploid cells and Walters and Gerstel reported triple and quadruple terminal chiasmata in the spontaneous tetraploid. Double chiasmata (Figure 11*A*), but not triple and quadruple chiasmata, were observed in the present study. Higher chiasmata numbers associating homologous segments were absent, presumably because of chiasmata interference and the spatial separation of homologous segments in the nucleus prior to synapsis.

As established by Kato,[6] the chromosomes of diploid *Rhoeo* occupy an exact position relative to one another in the ring or chain, due to chiasmata formation between homologous segments. In the tetraploid, where homologous segments are present in quadruplicate, the position of the chromosomes relative to one another is not fixed.

Summary

A cytological study of two colchicine induced tetraploid plants of *Rhoeo discolor* Hance was made. Analysis of 202 microsporocytes revealed a variety of chromosome configurations consisting of chains, rings, pairs and univalents. Short chains were most frequently observed. The formation of pairs of chromosomes indicated that chiasmata are sometimes formed between completely homologous chromosomes although less frequently than between homologous segments.

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